

deoxynucleotidyl transferase (TdT) and poly A polymerase. When TdT or polyA polymerase are employed in the detection step, the second oligonucleotide may contain a 5' end label. The invention is not limited by the nature of the 5' end label; a wide variety of suitable 5' end labels are known to the art and include biotin, fluorescein, tetrachlorofluorescein, hexachlorofluorescein, Cy3 amidite, Cy5 amidite and digoxigenin.

The novel detection methods of the invention may be employed for the detection of target DNAs and RNAs including, but not limited to, target DNAs and RNAs comprising wild type and mutant alleles of genes, including genes from humans or other animals that are or may be associated with disease or cancer. In addition, the methods of the invention may be used for the detection of and/or identification of strains of microorganisms, including bacteria, fungi, protozoa, ciliates and viruses (and in particular for the detection and identification of RNA viruses, such as HCV).

DESCRIPTION OF THE DRAWINGS

Figure 1A provides a schematic of one embodiment of the detection method of the present invention.

Figure 1B provides a schematic of a second embodiment of the detection method of the present invention.

Figure 2 is a comparison of the nucleotide structure of the DNAP genes isolated from *Thermus aquaticus* (SEQ ID NO:1), *Thermus flavus* (SEQ ID NO:2) and *Thermus thermophilus* (SEQ ID NO:3); the consensus sequence (SEQ ID NO:7) is shown at the top of each row.

Figure 3 is a comparison of the amino acid sequence of the DNAP isolated from *Thermus aquaticus* (SEQ ID NO:4), *Thermus flavus* (SEQ ID NO:5), and *Thermus thermophilus* (SEQ ID NO:6); the consensus sequence (SEQ ID NO:8) is shown at the top of each row.

Figures 4A-G are a set of diagrams of wild-type and synthesis-deficient DNAP_{Taq} genes.

Figure 5A depicts the wild-type *Thermus flavus* polymerase gene.

Figure 5B depicts a synthesis-deficient *Thermus flavus* polymerase gene.

Figure 6 depicts a structure which cannot be amplified using DNAP*Taq*.

Figure 7 is a ethidium bromide-stained gel demonstrating attempts to amplify a bifurcated duplex using either DNAP*Taq* or DNAPStf (*i.e.*, the Stoffel fragment of DNAP*Taq*).

Figure 8 is an autoradiogram of a gel analyzing the cleavage of a bifurcated duplex by DNAP*Taq* and lack of cleavage by DNAPStf.

Figures 9A-B are a set of autoradiograms of gels analyzing cleavage or lack of cleavage upon addition of different reaction components and change of incubation temperature during attempts to cleave a bifurcated duplex with DNAP*Taq*.

Figures 10A-B are an autoradiogram displaying timed cleavage reactions, with and without primer.

Figures 11A-B are a set of autoradiograms of gels demonstrating attempts to cleave a bifurcated duplex (with and without primer) with various DNAPs.

Figures 12A shows the substrates and oligonucleotides used to test the specific cleavage of substrate DNAs targeted by pilot oligonucleotides.

Figure 12B shows an autoradiogram of a gel showing the results of cleavage reactions using the substrates and oligonucleotides shown Fig. 12A.

Figure 13A shows the substrate and oligonucleotide used to test the specific cleavage of a substrate RNA targeted by a pilot oligonucleotide.

Figure 13B shows an autoradiogram of a gel showing the results of a cleavage reaction using the substrate and oligonucleotide shown in Fig. 13A.

Figure 14 is a diagram of vector pTTQ18.

Figure 15 is a diagram of vector pET-3c.

Figure 16A-E depicts a set of molecules which are suitable substrates for cleavage by the 5' nuclease activity of DNAPs.

Figure 17 is an autoradiogram of a gel showing the results of a cleavage reaction run with synthesis-deficient DNAPs.

Figure 18 is an autoradiogram of a PEI chromatogram resolving the products of an assay for synthetic activity in synthesis-deficient DNAP*Taq* clones.

Figure 19A depicts the substrate molecule used to test the ability of synthesis-deficient DNAPs to cleave short hairpin structures.

Figure 19B shows an autoradiogram of a gel resolving the products of a cleavage reaction run using the substrate shown in Fig. 19A.

Figure 20A shows the A- and T-hairpin molecules used in the trigger/detection assay.

Figure 20B shows the sequence of the alpha primer used in the trigger/detection assay.

Figure 20C shows the structure of the cleaved A- and T-hairpin molecules.

Figure 20D depicts the complementarity between the A- and T-hairpin molecules.

Figure 21 provides the complete 206-mer duplex sequence employed as a substrate for the 5' nucleases of the present invention

Figures 22A and B show the cleavage of linear nucleic acid substrates (based on the 206-mer of Figure 21) by wild type DNAPs and 5' nucleases isolated from *Thermus aquaticus* and *Thermus flavus*.

Figure 23 provides a detailed schematic corresponding to the of one embodiment of the detection method of the present invention.

Figure 24 shows the propagation of cleavage of the linear duplex nucleic acid structures of Figure 23 by the 5' nucleases of the present invention.

Figure 25A shows the "nibbling" phenomenon detected with the DNAPs of the present invention.

Figure 25B shows that the "nibbling" of Figure 25A is 5' nucleolytic cleavage and not phosphatase cleavage.

Figure 26 demonstrates that the "nibbling" phenomenon is duplex dependent.

Figure 27 is a schematic showing how "nibbling" can be employed in a detection assay.

Figure 28 demonstrates that "nibbling" can be target directed.